REMARKS

Support for Claim Amendments

The term "skin" is supported in the specification on page 3, paragraphs 2-4; page 5, starting on line 15; and page 15, lines 31-32. Skin is also defined on page 15. Descriptions of applying pressure against the skin to occlude blood vessels are present on pages 3 and 5, as well as in the examples. Specifically, the use of a cuff applied over the skin is found in example 1 on page 23, and in example 10 on page 32.

"9 7" does not exist in claim 11. The numeral 9 has a strike though it indicating deletion of this number from the original claim. The numeral 7 was mistakenly not underlined to indicate addition of the numeral to the amended claim. This amendment served to make the claim dependent from claim 7 rather than the canceled claim 9.

The abbreviation for the term "profundis", prof., is found on page 26, Table 1A. The abbreviation was amended to the unabbreviated word at the request of the Examiner in the Office Action dated March 11, 2003, page 8, lines 8-9.

The word "distal" is a common term used to describe location relative to a point or origin and does not include new matter. Distal is defined in standard, scientific, and medical dictionaries as: *1. situated away from the point of attachment or origin or a central point especially of the body; 2. (anatomy) directed away from the midline or mesial plane of the body; 3. farther from a point of reference, opposed to proximal; 4. away from point of attachment: used to describe a body part situated away from a point of origin. For example, the elbow is distal to the shoulder; 5. describes a feature anatomically located farther away from, or in the direction away from, the central part of the body or point of attachment or origin.* The term "distal to" appears on page 32, line 19 in the same context as is used in claim 39.

Applicants previously provided support in the response dated May 9, 2003, for claim 39 "wherein inserting the polynucleotide, applying pressure, and expressing the polynucleotide does not diminish use of the limb by the mammal." Support can be found on page 22, lines 15-27; page 25 lines 17-25, page 28, lines 6-23. Applicants recognize that it is important that a gene delivery procedure not cause harm to the recipient, such as a gene therapy patient. As such, Applicants examined the possibility that the described delivery procedure caused damage to the limb either through ischemia induced by vessel occlusion or edema caused by injection of the polynucleotide-containing solution. Applicants have shown that the delivery procedure does not cause damage to the limb or result in decreased use of the limb in primate, rat, and mouse.

Within the specification, Applicants have clearly stated that: no ischemic damage was observed in the tissue following the procedure, there was no evidence of damage to nerves in the limbs, the animals tolerated the procedure well, no discomfort was caused to the animals beyond normal surgical recovery, serum enzyme levels (a measure of toxicity) were minimally elevated and returned to normal quickly, and there was no histological sign of pathology. A limb muscle gene therapy procedure that causes damage to a limb would be undesirable. Applicants' believe that the phrase "wherein inserting the polynucleotide, applying pressure, and expressing the polynucleotide does not diminish use of the limb by the mammal" is a supported component of the claim.

The term "repetitive" appears on page 30 line 1. An example of repetitive administration of immunosuppressive drugs, as used in claim 40, is found in the specification in: example 5 (page 28, line 29 continuing to page 30, line 2) and example 9 (page 31, lines 31-32). In each of these examples, immunosuppressive drugs were administered once a day for the duration of the experimental analysis. Applicants have demonstrated delivery of polynucleotides to cells without administering immunosuppressive drugs, with administering 2-3 injections of immunosuppressive drugs and with daily injections of immunosuppressive drugs. Therefore, Applicants' use of "repetitive" or "single" administration does not constitute new matter.

The rationale for transient or long term immunosuppression in relation to gene therapy is well established in the art. The desired outcome of gene therapy may be the short or long term expression of a protein that was not previously expressed as an endogenous protein in the patient. Because the transgenic protein may not be an endogenous protein, there arises the potential of an immune response against the transgenic protein. An analogous situation arises in organ transplantation. Rejection of the organ is an immune response which is suppressed by the administration of immunosuppressive drugs. Patients who receive organ transplants often remain on immunosuppressive treatment for years or even for the rest of their lives. Rejection of a transgenic gene product and the cells expressing the transgenic gene would be treated in a similar manner. Immunosuppressive drugs may have to be given to the patient for as long as the transgene is expressed or as long as is necessary to build tolerance to the product. This type of immunosuppressive treatment is readily recognized as long term or repetitive immunosupressive treatment.

Acute conditions, such as severe allergic reactions, anaphylactic shock, and certain toxins are also treated with immunosuppressive drugs. It is well known in the field of gene therapy that immune response to a component of the delivery system may also be a potential limiting factor in effective gene delivery. Short term, or transient, immunosuppression may be required for efficient gene therapy in such cases. There is extensive knowledge in the use of immunosuppression for both chronic and acute medical conditions and the level of skill in the art is high. Therefore, no undue experimentation would be required by those skilled in the art to understand when and what type of immunosuppressive treatment is necessary for the claimed process. Applicants demonstrated the potential benefits of immunosuppression in enhancing gene therapy in example 5 of the specification (page 28-29). The observed increased expression over a longer time period can reasonably be considered the result of suppression of an immune response to the transgenic gene product.

Rejection of claims under 35 U.S.C. 112

Claims 1-3, 5-7, 11, 12, 16-20, 24, 25, 27, 27-31, 34-36 and 38-42 have been rejected under U.S.C. 112 first and second paragraph. Applicants have amended the claims to obviate the rejections. The amended claims 1 and 39 more clearly indicate the muscle cells to which the nucleic acid is delivered in relation to the vessel into which the nucleic acid is inserted. The amended claims also more clearly indicate which vessels are occluded by the applied pressure.

Methods for occluding blood flow by applying external pressure are well known in the art. Tourniquets are a well known method of stopping blood loss in emergency medical situations. Sphygmomonameters, used to measure blood pressure, measure the pressure (applied externally against the skin) needed to stop blood flow to a limb. Pressure points, places where one applies external pressure to compress internal vessels to occlude blood flow

and prevent blood loss, are taught in first aid classes. Numerous devices can be engineered to apply pressure to the skin to prevent blood flow.

Applicants' process should not be limited to one type of device for applying external pressure. The novelty of the invention does not reside with such a device but resides in the non-invasive application of external blood vessel compression to facilitate polynucleotide delivery. Thus, Applicants have taught applying external pressure to the limb.

On page 5, the Action states that Applicants' claims encompass Milas *et al.* The method taught by the Applicants is differentiated from the method taught by Milas et al 1997 in three distinct ways. First, Fig. 1A of Milas *et al.* is a "schematic depiction of isolated limb perfusion." The definition of schematic from the Cambridge Dictionary: showing the main form and features of something, usually in the form of a drawing, which helps people to understand it. The text on page 2198, column 2 indicates that the tourniquet, although schematically represented as around the rat limb is in fact placed "underneath the inguinal ligament." Second, the method of Milas et al teach perfusion of the limb requiring surgical cannulation of two vessels. Milas et al further teach perfusion with normal saline both before and after perfusion of the adenovirus. Milas et al state on page 2202, paragraph 1 that "cannulation of the femoral vein with resultant brisk outflow is critical for the success of the procedure… ." Finally, Milas et al teach the ligation of the vessels distal to the cannulation sites, page 2199, paragraph 1. In contrast Applicants teach injection into a single blood vessel and a non-invasive cuff without ligation of the vessel after the procedure. Applicants believe that the amended claims do not encompass the methods taught by Milas and Ye.

The Office Action states on page 5 that it can not be determined how delivery to specific muscle cells (claims 11, 12, 17, 24, 25, and 29-31) is affected by the location of the blood vessel injected. Applicants respectfully disagree.

The specification indicates that the polynucleotide is injected into an afferent or efferent vessel of the target tissue or organ. In other words, the vessel is selected by identifying the vessel or vessels that deliver blood to or carry blood away from the target tissue. Applicants furthermore believe that claims 11, 12, 17, 24, 25, and 29-31 are not restricting delivery to the explicitly named muscle cells. The named muscles are present in the arm or leg and therefore practice of the invention results in delivery to the claimed muscle cells.

For example, the iliac artery supplies blood to all the major muscle groups of the leg. Applicants have shown that injection into the iliac artery results in polynucleotide delivery to cells of all the major muscle groups of the leg. The popliteal artery supplies blood to the lower leg. It would be expected that injection into the popliteal artery, with occlusion proximal to the insertion point, would result in delivery of polynucleotides to muscle cells in the lower leg, but not the thigh. Applicants demonstrated, in example 10 on page 32 of the specification, that placement of the tourniquet (i.e. vessel occlusion) results in delivery of the polynucleotide to muscle cells distal to the vessel occlusion, but not proximal to the occlusion. Applicants furthermore believe that claims 11, 12, 17, 24, 25, and 29-31 do not restrict delivery to only the explicitly named muscle cells. The named muscles are present in the arm or leg and therefore practice of the invention may result in delivery to the claimed muscle cells depending on the vessel into which the polynucleotide is inserted, the point of injection into the vessel and the location of vessel occlusion.

The Office Action states on page 6 that the specification does not correlate the results obtained with naked plasmid DNA to any other vector. Applicants have submitted data with their previous Response demonstrating a correlation. These examples, using a similar process, demonstrate delivery of DNA/polycation complexes, small double stranded RNA oligonucleotides, and viral particles.

The Office Action states on page 6 that Applicants' specification teaches only delivery of a polynucleotide linked to a promoter and not to any polynucleotide as broadly claimed. The submitted data provide a large number of different polynucleotides which reasonably support Applicants' use of the general term "polynucleotide."

The Office Action states on page 8 that "the mere delivery of polynucleotide to any cell does not have a disclosed use after delivering the DNA to a skeletal muscle cell. The method should result in expression of a protein in skeletal muscle cell." Applicants respectfully disagree.

Applicants point to the extensive evidence in the scientific literature of nucleic acid delivery to a cell for the purpose of inhibiting expression of an endogenous gene (see reviews: Wang et al. Antisense anticancer oligonucleotide therapeutics. Curr Cancer Drug Targets 2001 1(3):177-196; Mercatante et al. Modification of alternative splicing by the antisense oligonucleotides as a potential chemotherapy for cancer and other diseases. Curr Cancer Drug Targets 2001 1(3):211-230; Cho-Chung. Antisense DNAs as targeted therapeutics for cancer: no longer a dream. Curr Opin Investig Drugs 2002 3(6):934-939; Shuey et al. RNAi: gene-silencing in therapeutic intervention. Drug Discov Today 2002 7(20):1040-1046; and Shi. Mammalian RNAi for the masses. Trends Genet 2003 19(1):9-12). The delivered nucleic acid itself can inhibit expression of the endogenous gene as in antisense and RNA interference mechanisms of inhibition. Alternatively, the nucleic acid can be expressed to produce an RNA transcript that inhibits expression of an endogenous gene. In these examples, the delivered nucleic acid need not result in expression of a protein to alter the endogenous properties of the cell.

Claim 5 has been largely incorporated into claim 1 and has been canceled. Claims 6, 18, 19, 20, 24, 27, 28, and 30 have been amended to correct the indefinite rejections. Claims 11, 12, 16, 17, 34 and 35 have been amended to make them dependent on the appropriate claims. Claims 11, 12, 16, 17, 30, and 31 have been amended in response to the Markush group rejections. Claim 27 has been canceled, and claim 30 has been amended to depend from claim 6. Claims 11 and 12 have been amended to remove the abbreviations, spf. and prof.

The Office Action states on page 9 that the metes and bounds of "cuff" can not be determined. Applicants believe they have clearly defined a cuff, for the purposes of the claims, as: a device applied exterior to the mammal's skin that touches the skin in a non-invasive manner. As stated above, Applicants believe that the art of compressing blood vessels using pressure exerted against the surface of a mammal from an external position is well established. Therefore, Applicants believe that the metes and bounds of the term "cuff" are readily envisioned.

The Office Action states that the metes and bounds of claim 37 can not be determined. Applicants point out that claim 37 has previously been canceled.

The Office Action dated March 11, 2003 stated on page 10 that the metes and bounds of non-vascular parenchymal cells (claim 38) can not be determined because the specification does not define the parenchymal cells of vascular tissue. Applicants respectfully disagree. Applicants defined parenchymal cells as the distinguishing cells of a tissue. Therefore, cells such as smooth muscle cells may be considered blood vessel parenchymal cells. Similarly, endothelial cells may be considered blood vessel parenchymal cells. However, endothelial cells within capillaries of the liver would not be considered liver parenchymal cells since all tissues contain capillary endothelial cells. Since all cells of blood vessels are vascular cells, and Applicants state non-vascular cells, the distinguishing cells of a blood vessel do not require a definition.

Rejection of claims under 35 U.S.C. 102

Claims 1, 3, 5, 6, 11, 12, 16, 17, 24, 25, 27-31, 34-39 and 38-42 have been rejected under 35 U.S.C. 102 as being anticipated by Milas et al 1997. Applicants have amended the claims to obviate the rejections. Applicants believe that the amended claims are distinguished from the method taught by Milas et al. for the reasons stated in response to the §112 rejections. Importantly, Milas et al. do not deliver to muscle as stated on page 2201, 3rd full paragraph.

The Office Action states on page 12 that occluding blood vessels is immunosuppression because blood cells are prevented from flowing through the area. However, since the tissues are not perfused to remove blood cells, immune cells remain present in the area and the act of occluding blood flow does not constitute immunosuppression. As discussed above, the use of immunosuppression in medical procedures is common.

Claims 1-42 are rejected as being anticipated by Von der Leyen et al 1999. Applicants have amended to claims to more distinctly differentiate their method from the method of Von der Leyen. Applicants note, however, that a sphygmomanometer was used by Von der Leyer, not to increase pressure in the artery, but to monitor pressure in an isolated arterial segment. The sphygmomanometer was not placed around a limb. Furthermore, while Von der Leyen did observe delivery of nucleic acid to multiple layers of the artery (page 2362, column 1, lines 16-21) which is surrounded by skeletal muscle in vivo, they did not observe delivery out of the vessel to skeletal muscle cells.

Rejection of claims under 35 U.S.C. 103

Claims 1, 3, 5, 6, 11, 12, 16, 17, 27, 28, 30, 31, 34-36 and 38-42 have been rejected under 35 U.S.C. 103(a) as being unpatentable over Wolff '387 in view of Milas et al 1977. Claims 1, 3, 5, 6, 11, 12, 16, 17, 24, 25, 27-31, 34-36 and 38-42 have been rejected under 35 U.S.C. 103(a) as being unpatentable over Budker et al. 1998 in view of Milas et al 1977. Applicants have shown that Milas et al. does not utilize a device over the skin. Therefore, the rejection is believed to be overcome.

Claims 1, 3, 5, 6, 11, 12, 16, 17, 24, 24, 27-31, 34-36 and 38-42 have been rejected under 35 U.S.C. 103(a) as being unpatentable over Milas et al 1977 in view of Nabel '488. Applicants have amended the claims to obviate the rejections. Applicants believe that the amended claims do not encompass the method taught by Milas et al. for the reasons stated in response to the §112 rejections.

Double patenting

For the reasons stated in this Response, Applicants' have shown that Milas et al. does not teach or show the non-invasive application of a tourniquet over skin to impede blood flow. Therefore, this rejection is believed to be obviated.

The Examiner's objections and rejections are now believed to be overcome by this response to the Office Action. In view of Applicants' amendment and arguments, it is submitted that claims 1-3, 6, 7, 11-12, 16-20, 24, 25, 28-31, 34-36 and 38-42 should be allowable and Applicants respectfully request an early notice to such effect.

Respectfully submitted,

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I hereby certify that this correspondence is being sent by facsimile transmission to: (703) 308-4242, Commissioner for Patents, P.O. Box 1450, Alexandria, VA 22313-1450; in this date:

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